



# UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE  
United States Patent and Trademark Office  
Address: COMMISSIONER FOR PATENTS  
P.O. Box 1450  
Alexandria, Virginia 22313-1450  
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/784,980	02/25/2004	Ekaterina Alekseevna Savrasova	US-164	8974

38108 7590 11/29/2005

CERMAK & KENEALY LLP  
ACS LLC  
515 EAST BRADDOCK ROAD  
SUITE B  
ALEXANDRIA, VA 22314

EXAMINER

FERNANDEZ, SUSAN EMILY

ART UNIT PAPER NUMBER

1651

DATE MAILED: 11/29/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

<b>Office Action Summary</b>	<b>Application No.</b> 10/784,980	<b>Applicant(s)</b> SAVRASOVA ET AL.	
	<b>Examiner</b> Susan E. Fernandez	<b>Art Unit</b> 1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

#### Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

#### Status

- 1) ☒ Responsive to communication(s) filed on 09 August 2005.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

#### Disposition of Claims

- 4) ☒ Claim(s) 1-13 is/are pending in the application.
- 4a) Of the above claim(s) \_\_\_\_\_ is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 1-13 is/are rejected.
- 7) ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

#### Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on \_\_\_\_\_ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.  
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).  
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

#### Priority under 35 U.S.C. § 119

- 12) ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☒ All b) ☐ Some \* c) ☐ None of:
- ☒ Certified copies of the priority documents have been received.
  - ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
  - ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\* See the attached detailed Office action for a list of the certified copies not received.

#### Attachment(s)

- |  |   |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892)  | 4) <input type="checkbox"/> Interview Summary (PTO-413)<br>Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948)                                   | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152)             |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08)<br>Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____  |

### DETAILED ACTION

The amendment filed August 9, 2005, has been received and entered. The text of those sections of Title 35, U.S. Code, not included in this action can be found in prior office action.

Claims 1-13 are pending and are presented for examination.

#### *Claim Rejections - 35 USC § 103*

Claims 1, 4, 10, and 11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tsuchida et al. (JP 360047692).

Tsuchida et al. teaches the cultivation of three strains of *Escherichia coli* for the production of L-threonine in high yield. Because there is a high yield of L-threonine, the bacterium has enhanced expression of genes for L-threonine biosynthesis. The culture medium contains xylose and glucose. See translated abstract.

Tsuchida et al. does not expressly disclose the ratio of glucose to pentose sugars.

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to have used various ratios of glucose to pentose sugars in the culture medium disclosed by Tsuchida et al., including ratios within the ratio range recited in claim 1 under examination. The selection of a specific suitable glucose to pentose sugar range, including that claimed, clearly would have been an obvious matter of optimization on the part of the artisan of ordinary skill. Moreover, Tsuchida et al. does not disclose any ranges which would not have been suitable.

Applicant's arguments have been fully considered but they are not persuasive. First, applicants assert that Tsuchida et al. does not teach/suggest all elements of the claimed invention

Art Unit: 1651

by first pointing out that Tsuchida et al. does not disclose L-amino acid production using a mixture of glucose and pentose as the main carbon source. Additionally, applicants urge that Tsuchida et al. does not teach the claimed invention since the method of the instant invention does not require lactose or galactose as an essential carbon source, as disclosed by Tsuchida et al. However, the claims under examination do not recite that a mixture of glucose and pentose is the main carbon source, or provide any limitations as to the main carbon source for that matter. Instead, it is respectfully noted that the claims under examination require that "culture medium contains a mixture of glucose and pentose sugars...", and this recitation clearly does not exclude other ingredients, such as lactose or galactose, from inclusion in the medium. Tsuchida et al. clearly teaches a culture medium which contains a mixture of glucose and pentose sugars, where the pentose sugar is xylose (see also page 3 of English translation of '692). Because the cited prior art suggests all the presently claimed steps, a holding of obviousness is required.

Claims 1-5, and 10-11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tsuchida et al. in view of Nichols et al. (Applied Microbiol Biotechnol, 2001, 56: 120-125) or Aristidou et al. (Current Opinion in Biotechnology, 2000, 11: 187-198).

Tsuchida et al. teaches the cultivation of three strains of *Escherichia coli* for the production of L-threonine in high yield. Because there is a high yield of L-threonine, the bacterium has enhanced expression of genes for L-threonine biosynthesis. The culture medium contains xylose and glucose. See translated abstract.

Tsuchida et al. does not expressly disclose a culture medium containing arabinose in addition to xylose and glucose, or that the mixture of sugars is a feedstock mixture of sugars

Art Unit: 1651

obtained from cellulosic biomass. Furthermore, it does not teach using a bacterium modified for increased rate of pentose sugar utilization.

Nichols et al. and Aristidou et al. teach the construction of *E. coli* strains which grow in hemicellulose hydrosylates and produce ethanol. See the abstract, and first two paragraphs under "Discussion" on page 124 of Nichols et al., and the "Escherichia coli" section on pages 190 and 191 of Aristidou et al. Furthermore, the cellulosic biomasses for *E. coli* cultivation comprise of glucose, arabinose, and xylose (page 124, first paragraph under "Discussion" of Nichols et al., page 190, first paragraph under "Escherichia coli" of Aristidou). Moreover, Nichols et al. and Aristidou et al. both teach the use of *E. coli* strains engineered for enhanced pentose sugars utilization. In particular, Nichols et al. constructed the *ptsG* mutant FBR14 which ferments arabinose and xylose simultaneously with glucose, unlike parent strain FBR5 (page 124, third paragraph under "Discussion"). Aristidou et al. discusses a recombinant strain of *E. coli* capable of fermenting glucose first, then arabinose and xylose (page 190, second column, first full paragraph).

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to have used the cellulosic biomass as discussed in Nichols et al. and Aristidou et al. as the culture media for practicing the Tsuchida invention. Furthermore, it would have been obvious to have engineered the *E. coli* strains of the Tsuchida invention for increased rate of pentose sugars utilization.

One of ordinary skill in the art would have been motivated to do this because the use of abundant renewable resources such as cellulosic biomass for ethanol production is highly desirable. Aristidou states that "ethanol is a versatile transportation fuel that offers high octane,

Art Unit: 1651

high heat of vaporization, and other characteristics that allow it to achieve higher efficiency use in optimized engines than gasoline” (page 188, first column, first full paragraph). Thus the production of ethanol in addition to amino acids would have been desired, and cellulosic biomass would have served as a less expensive alternative to other sources of sugars. Additionally, modifying the bacterium in order to increase the rate of pentose sugars utilization would have increased the yield of amino acids from pentose sugars, thus optimizing the process. A holding of obviousness is therefore proper.

Applicant's arguments have been fully considered but they are not persuasive. As discussed above, the Tsuchida invention clearly suggests the culture medium as recited in parent claim 1, contrary to applicants' assertions. Applicants further suggest that there is no motivation to combine Nichols et al. and/or Aristidou et al. with the teachings of Tsuchida et al. since Nichols et al. and Aristidou et al. are concerned with the production of ethanol via fermentation, which is involved with a different pathway than that of amino acids. Though the production of ethanol and amino acids are different, this does mean that the production of the two cannot be concurrent. As pointed out above, there is motivation to produce ethanol **in addition** to amino acids. The use of the same medium and *E.coli* organisms for the production of both would have been highly desirable, since it would have allowed for the production of multiple useful products from a single culture, instead of multiple cultures of *E. coli*. Since there is motivation to combine the references, the rejections over the prior art are proper.

Claims 1-7 and 10-11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nichols et al. in view of Hashiguchi et al. (US Pat. 5,998,178).

Nichols et al. discloses the growth of *E. coli* strains in hemicellulose hydrosylates in order to produce ethanol. See the abstract, and first two paragraphs under "Discussion" on page 124 of Nichols et al. Furthermore, the cellulosic biomass for *E. coli* cultivation comprises of glucose, arabinose, and xylose (page 124, first paragraph under "Discussion" of Nichols et al.). Moreover, Nichols et al. teaches the use of *E. coli* strains engineered for enhanced use of pentose sugars utilization. Various strains were examined, including W3110 (Table 1, page 121). The W3110 strain was transformed with the *pet* plasmid, pLOI297, which resulted in increased utilization of xylose when grown in media consisting of xylose and glucose (page 122, first column, first full paragraph, and Fig. 1a, b). Furthermore, a *ptsG* mutant FBR14 was constructed which ferments arabinose and xylose simultaneously with glucose, unlike parent strain FBR5 (page 124, third paragraph under "Discussion").

Nichols et al. does not expressly disclose the production of L-isoleucine, or specify the glucose to xylose ratio range recited in claim 1 under examination.

Hashiguchi et al. discloses preparing an L-isoleucine-producing bacterium. Furthermore, Hashiguchi et al. notes that "L-isoleucine can be prepared in good efficiency by cultivating a bacterium belonging to the genus *Escherichia* which is transformed by incorporating the released type thrABC operon prepared above and which further carries the released type ilvGMEDA operon in a suitable medium to thus produce L-isoleucine and accumulate it in the culture medium" (column 23, lines 51-58). *E. coli* W3110 strain is included as an example (column 16, lines 61-66).

Art Unit: 1651

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to have transformed the W3110 strain discussed in Nichols et al. in the same manner as disclosed in Hashiguchi et al. in order to yield L-isoleucine.

One of ordinary skill in the art would have been motivated to do this because Hashiguchi et al. states that L-isoleucine is “essential for human and other animals and is principally useful as a material for various drugs represented by a medicine for promoting nutrition (nutrient)” (column 1, lines 8-11). It would have been obvious to have enhanced isoleucine production in ethanol-producing *E. coli*. Furthermore, the selection of a specific suitable glucose to pentose sugar range, including that claimed, clearly would have been an obvious matter of optimization on the part of the artisan of ordinary skill.

All of applicants’ arguments have been fully considered but are not persuasive of error. While strain IT1168 instead of strain W3110 showed increased utilization of xylose for ethanol production, Nichols et al. still discloses the growth of *E. coli* strains in hemicellulose hydrolysates where the cellulosic biomass for *E. coli* cultivation comprises of glucose, arabinose, and xylose. Additionally, as discussed above, there is sufficient motivation to combine the Nichols reference with Hashiguchi et al. since the production of ethanol in addition to amino acids would have been desirable, and their concurrent production clearly would have been more efficient than their production from separate cultures. Thus, the rejections are properly maintained.

Claims 1-5, and 8-9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Deabov et al. (RU 2003677C) in view of Nichols et al.



Deabov et al. discloses a strain of *Escherichia coli* capable of producing a higher yield of L-histidine. See DERWENT abstract.

Deabov et al. does not expressly disclose a culture medium for *E.coli* cultivation comprising of arabinose, xylose, and glucose at a particular ratio range, or that the mixture of sugars in the medium is obtained from cellulosic biomass. Furthermore, the modification of the L-histidine-producing bacterium in order to increase the rate of pentose sugars utilization is not disclosed.

Nichols et al. discloses the growth of *E. coli* strains in hemicellulose hydrosylates in order to produce ethanol. See the abstract, and first two paragraphs under "Discussion" on page 124 of Nichols et al. Furthermore, the cellulosic biomass for *E. coli* cultivation comprises of glucose, arabinose, and xylose (page 124, first paragraph under "Discussion" of Nichols et al.). Moreover, Nichols et al. teaches the use of *E.coli* strains engineered for enhanced use of pentose sugars utilization. Various strains were examined, including W3110 (Table 1, page 121). The W3110 strain was transformed with the *pet* plasmid, pLOI297, which resulted in increased utilization of xylose when grown in media consisting of xylose and glucose (page 122, first column, first full paragraph, and Fig. 1a, b). Furthermore, a *ptsG* mutant FBR14 was constructed which ferments arabinose and xylose simultaneously with glucose, unlike parent strain FBR5 (page 124, third paragraph under "Discussion").

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to have used the cellulosic biomass as discussed in Nichols et al. as the culture media for practicing the Deabov invention. Furthermore, it would have been obvious to have

Art Unit: 1651

engineered the *E.coli* strains of the Deabov invention for increased rate of pentose sugars utilization.

One of ordinary skill in the art would have been motivated to do this because the use of abundant renewable resources such as cellulosic biomass for ethanol production is highly desirable. Ethanol can be used as an efficient fuel for automobiles. Thus the production of ethanol in addition to amino acids would have been desired, and cellulosic biomass would have served as a less expensive alternative to other sources of sugars. Additionally, modifying the bacterium in order to increase the rate of pentose sugars utilization would have increased yield of amino acids from pentose sugars, thus optimizing the process. Furthermore, the selection of a specific suitable glucose to pentose sugar range, including that claimed, clearly would have been an obvious matter of optimization on the part of the artisan of ordinary skill.

Applicant's arguments have been fully considered but they are not persuasive. Applicants assert that Deabov et al. does not teach a culture medium with the proper components which allow superior production of amino acid. However, the claims under examination do not recite that the components in the culture medium allow for superior production of amino acids, and instead require the cultivation of an L-amino acid-producing bacterium in a culture medium which contains a mixture of glucose and pentose sugars of a certain ratio range. Any culture medium which contains this particular mixture of sugars would have satisfied the claims as long as the medium allows for L-amino acid-producing bacterium cultivation. Nichols et al. cures the deficiencies of Deabov et al. (presence of glucose and pentose sugars in culture medium). As discussed above, there would have been sufficient motivation to combine the references since the concurrent production of ethanol and amino acid from a single culture would have been

Art Unit: 1651

desirable, yielding a more efficient manner of producing two needed products which have different pathways.

Claims 1-5 and 10-11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nichols et al. in view of Liaw et al. (US 2002/0106800).

Nichols et al. the growth of *E. coli* strains in hemicellulose hydrosylates in order to produce ethanol. See the abstract, and first two paragraphs under "Discussion" on page 124 of Nichols et al. Furthermore, the cellulosic biomass for *E. coli* cultivation comprises of glucose, arabinose, and xylose (page 124, first paragraph under "Discussion" of Nichols et al.). Moreover, Nichols et al. teaches the use of *E. coli* strains engineered for enhanced pentose sugars utilization. Various strains were examined, including W3110 (Table 1, page 121). The W3110 strain was transformed with the *pet* plasmid, pLOI297, which resulted in increased utilization of xylose when grown in media consisting of xylose and glucose (page 122, first column, first full paragraph, and Fig. 1a, b). Furthermore, a *ptsG* mutant FBR14 was constructed which ferments arabinose and xylose simultaneously with glucose, unlike parent strain FBR5 (page 124, third paragraph under "Discussion").

Nichols et al. does not expressly disclose the production of L-threonine, or the ratios of glucose to pentose sugars recited in instant claim 1.

Liaw et al. discloses the preparation of *E. coli* ADM Kat26, which is obtained by infecting *E. coli* W3110 strain with a P1 lysate. See page 17, paragraph 0237. This strain is capable of producing threonine.

Art Unit: 1651

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to have infected the W3110 strain discussed in Nichols et al. with the P1 lysate of Liaw et al. in order to allow for threonine production.

One of ordinary skill in the art would have been motivated to do this because amino acids such as threonine may be used in food additives and medicines. It would have been obvious to have enhanced threonine production in ethanol-producing *E. coli*. Furthermore, the selection of a specific suitable glucose to pentose sugar range, including that claimed, clearly would have been an obvious matter of optimization on the part of the artisan of ordinary skill.

Applicant's arguments have been fully considered but they are not persuasive. As discussed above, though ethanol and amino acid production is different, the concurrent production of these two desired products from a single culture instead of from multiple cultures would have been desirable. Thus, a holding of obviousness is clearly required.

Claims 1-5, and 8-9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dunkak et al. (US Pat. 5,939,295) in view of Nichols et al.

Dunkak et al. discloses a strain of *Escherichia coli* capable of producing tryptophan. See claims 1-11 and the abstract.

Dunkak et al. does not expressly disclose a culture medium for *E. coli* cultivation comprising of arabinose, xylose, and glucose, or that the mixture of sugars in the medium is obtained from cellulosic biomass. Furthermore, the modification of the L-tryptophan-producing bacterium in order to increase the rate of pentose sugars utilization is not disclosed.

Nichols et al. discloses the growth of *E. coli* strains in hemicellulose hydrosylates in order to produce ethanol. See the abstract, and first two paragraphs under "Discussion" on page 124 of Nichols et al. Furthermore, the cellulosic biomass for *E. coli* cultivation comprises of glucose, arabinose, and xylose (page 124, first paragraph under "Discussion" of Nichols et al.). Moreover, Nichols et al. teaches the use of *E. coli* strains engineered for enhanced pentose sugars utilization. Various strains were examined, including W3110 (Table 1, page 121). The W3110 strain was transformed with the *pet* plasmid, pLOI297, which resulted in increased utilization of xylose when grown in media consisting of xylose and glucose (page 122, first column, first full paragraph, and Fig. 1a, b). Furthermore, a *ptsG* mutant FBR14 was constructed which ferments arabinose and xylose simultaneously with glucose, unlike parent strain FBR5 (page 124, third paragraph under "Discussion").

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to have used the cellulosic biomass as discussed in Nichols et al. as the culture media for practicing the Dunkak invention. Furthermore, it would have been obvious to have engineered the *E. coli* strains of the Dunkak invention for increased rate of pentose sugars utilization.

One of ordinary skill in the art would have been motivated to do this because the use of abundant renewable resources such as cellulosic biomass for ethanol production is highly desirable. Ethanol can be used as an efficient fuel for automobiles. Thus the production of ethanol in addition to amino acids would have been desired, and cellulosic biomass would have served as a less expensive alternative to other sources of sugars. Additionally, modifying the bacterium in order to increase the rate of pentose sugars utilization would have increased yield of

Art Unit: 1651

amino acids from pentose sugars, thus optimizing the process. Furthermore, the selection of a specific suitable glucose to pentose sugar range, including that claimed, clearly would have been an obvious matter of optimization on the part of the artisan of ordinary skill.

Applicant's arguments have been fully considered but they are not persuasive. As discussed above, though the production of ethanol and amino acids follow different pathways, their concurrent production would have been possible and desirable, thus requiring the use of a single culture to obtain the desired products rather than multiple ones. A holding of obviousness is clearly required.

No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a).

Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire **THREE MONTHS** from the mailing date of this action. In the event a first reply is filed within **TWO MONTHS** of the mailing date of this final action and the advisory action is not mailed until after the end of the **THREE-MONTH** shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than **SIX MONTHS** from the date of this final action.

Art Unit: 1651


Any inquiry concerning this communication or earlier communications from the examiner should be directed to Susan E. Fernandez whose telephone number is (571) 272-3444. The examiner can normally be reached on Mon-Fri 8:30 am - 5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mike Wityshyn can be reached on (571) 272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Susan E. Fernandez  
Assistant Examiner  
Art Unit 1651

sef



FRANCISCO PRATS  
PRIMARY EXAMINER